Message

From: Detlef Knappe [knappe@ncsu.edu]
Sent: 7/19/2019 11:25:00 AM
To: David Muddiman [dcmuddim@ncsu.edu]
CC: Jane Hoppin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=userebcfc262]; Jeffrey Enders [jrenders@ncsu.edu]; Nadine Kotlarz [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=userc79d3fb6]; Strynar, Mark [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5a9910d5b38e471497bd875fd329a20a-Strynar, Mark]
Subject: Re: Hoppin PFC method

Sounds good, Dave!
BTW, we have retention time and spectra for HydroEVE, so it's not really a new compound. It's in all of our current methods now.
Best,
Detlef

On Fri, Jul 19, 2019 at 6:49 AM David Muddiman <dcmuddim@ncsu.edu> wrote:

> Hi Detlef,
>
> Sounds reasonable to me. It is important to have those in the center of the calibration curve, that is where you get the best precision of your measurements (as you probably know ;-)
>
> Its all good, I will have my students look into this today, we have a lot of info on structure / response and I am curious if that is what is driving this or if it is just sub-optimal for the labile compounds but still good enough to get the data that is needed. I think we can play with source parameters other than heat/temp as well, as energetic collisions also drive up internal energy in an ion and can cause problems.
>
> Happy Friday everyone!
> Dave
>
> On Fri, Jul 19, 2019 at 6:30 AM Detlef Knappe <knappe@ncsu.edu> wrote:
>
> > Hi Dave,
> > I think if we dose the same volume of SIL from the same dosing solution into standards, samples, and QCs, the exact concentration (purity) of the SIL is not that critical, don't you think? We dose internal standard at a level corresponding to the middle of our standard curve. The key is good accuracy and precision with the dosing.
> > From an untargeted perspective, it is important to run blanks both with and without internal standard to understand what's coming from the internal standard.
> > Thank you for taking a closer look at the ionization fundamentals.
> > Best,
> > Detlef
>
> On Fri, Jul 19, 2019 at 5:41 AM David Muddiman <dcmuddim@ncsu.edu> wrote:
>
> > P.S. I especially worry about sourcing materials (SIL standards) from different places since they all have their own purification strategies (chemical) and isotopic enrichment / purity determinations. That being said, if you just want solid data in the ballpark of the absolute, we would have to spend time worrying about that, we will just make the measurements with the assumption that the standards we secure have correct concentrations and are chemically and isotopically pure. We always track lot numbers for each set of experiments because even from the same company, same product, but different batch, there are

differences. And, I am 99% certain that if I ran a "pure" standard I would see other compounds, how much I don't know but talking with my synthetic friends, they said these PFAS are hard to purify.

On Fri, Jul 19, 2019 at 5:03 AM David Muddiman <dcmuddim@ncsu.edu> wrote:

Thanks Detlef et al.

I appreciate the background Starting at your last statement, Becca told me that her and Zach observed this. Very important.

Regarding re-inventing, I agree. Not doing that, what I meant was if we add a bunch of new PFAS onto the list for the blood samples (and by the way, Jeff is doing this work, not Becca), we will have to make sure the numbers we are reporting are accurate. I have been doing IDMS for a long long time and standards are not what they are always reported to be. In other words, in reality, using SIL standards is not so much absolute quantification and we would like it to be, it is relative to the standard. If the standard is not correct concentration, all data in those sample sets is comparable and precise but there is a risk of systematic error due to the standard not being correct. Jeff and I with Nadine's help will get Jane's samples looked at - my question pertained to that, not to stuff that your group is doing on their own / or with Becca. Sorry for any confusion on that front.

Regarding the high temp / low temp method, it is curious to me. I will have a look at these structures and do some calculations with my research group. We have also been studying biases in ESI since 1999. I am intriqued by the observations but I would like to understand their source. It could be high temp WOULD work best for everything but the labile species just can't handle it. That would make the most sense to me. Low Temp method would be so we don't fragment the labile species but in doing so, we reduce the signal.

Thanks everyone very much.

Dave

On Thu, Jul 18, 2019 at 9:56 PM Detlef Knappe <knappe@ncsu.edu> wrote:

Hi Dave,
The main consideration is sensitivity. The sulfonic acids ionize better at higher temp, giving us lower reporting limits. But at the higher temp, you obliterate the fluoroether carboxylic acids. So we have to run those at a lower temp. Lee Ferguson is seeing the same and is running both the low and high temp method to get the reporting limits we need. On an instrument with high sensitivity, it may be possible to just run at low temp, and I have asked Becca to check reporting limits she can get for all compounds using the low temp method. Please let us not reinvent things from scratch - we have been doing this for quite some time now. We need to make progress on samples. The days between now and July 29 are absolutely critical for getting results. If we spend more time on method development, we will be going to conferences in August and have nothing to report.
Another thing we just learned is that the branched ethers (PMPA, PEPA, GenX) are not stable in acetonitrile. We need to make all standards in methanol. And for carboxylic acids we need to use basic methanol to prevent the formation of methyl esters.
Best,
Detlef

On Thu, Jul 18, 2019 at 6:41 PM David Muddiman <dcmuddim@ncsu.edu> wrote:

Hi Mark

Perhaps you, James and my folks should have a talk about things. We are finding the analytical side of things to be strange. Does not make sense that compounds under gradient elution would have vastly

different desolation temperatures given the dominate factor is solvent comp. how can this be? There is something strange here. Need to figure out ASAP. In other words why don't the compounds at higher temp work at lower temps.

And big question why with "The Devil We Know" are we still studying this after 30 years. It is known there are health efforts from PFOS and GenX. Hmmmmm

Dave

Sent from my iPhone, Please forgive brevity and typos :-)

On Jul 18, 2019, at 6:24 PM, Jane Hoppin <jahoppin@ncsu.edu> wrote:

> Hi Dave
> I'm including Mark Strynar since the work was done in his lab, so I'm sure he'll have some thoughts about the solvent issue
>
> Cheers
>
> Jane
>
> On Thu, Jul 18, 2019 at 5:58 PM David Muddiman <dcmuddim@ncsu.edu> wrote:
> Hi Nadine,
>
> First, the low temp and high temp methods are curious to me. This should never be the case on a MS system. Something strange here going on. Solvent is solvent. So, while it might work, it does not make sense to me. I need to sort this out.
>
> Second, just adding this and that and this and that, means an entirely new method. We need to know what you want to measure. We can get compound with suspect concentrations and some with semi-reliable concentrations and "run the samples". We need to know what matter and do significant due diligence to make sure we can provide accurate numbers versus just numbers. So, the less we have to develop and QC/QA the sooner we can make this happen. Lots of samples and lots of analytes.
>
> Please advise, not just to Nadine but to Detlef and Jane too.
>
> Nadine, I saw you in Whole Foods yesterday but I knew I knew you but could not piece it together until your email. Safe travels,
> Dave
>
> On Thu, Jul 18, 2019 at 4:57 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:
> Thanks Nadine!
>
> On Thu, Jul 18, 2019 at 3:54 PM Nadine Kotlarz <nkotlar@ncsu.edu> wrote:
> Hi Jeff,
>
> We should start with, at a minimum, the 28 PFAS that are covered collectively on our Ultivo QQQ low temperature and high temperature methods. Here's the list

PFAS with standards from Chemours:

1. PFMOAA
2. PEPA
3. PMPA
4. PFO2HxA
5. PFO3OA
6. GenX
7. NVHOS
8. PFO4DA
9. Hydro-EVE
10. PFO5DoA
11. Nafion byproduct 1
12. Nafion byproduct 2
13. Nafion byproduct 4

PFAS with standards that can be purchased from Wellington:

1. PFBA
2. PFBS
3. PFPeA
4. PFPeS
5. PFHxA
6. PFHxS
7. PFHpA
8. PFHpS
9. PFOA
10. PFOS
11. PFNA
12. PFDA
13. 4:2FTS
14. 6:2FTS
15. 8:2FTS

We have some more standards from Chemours that didn't make it into the Ultivo method but may be good to incorporate into your method on the Altis. Those are the ones highlighted in blue in the attached doc.

We've also been using 20 internal standards for the analysis. We purchase one mix with 19 internal standards and MGenX separately. Invoice from a past purchase attached.

I'm out of town today and tomorrow but back in the office on Monday.
Nadine

On Thu, Jul 18, 2019 at 2:42 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:

> Hi Nadine,
> Can I get confirmation from you on the list provided below? I am trying to get these nailed down so that I can make sure we have all of the standards and then order the ones that we don't have and get started on method development. I am basing this list on

a table from the document attached. This document was given to Allison and is posted to this project on MENDIX. Thanks.

1	GenX
2	Nafionbp1
3	Nafionbp2
4	Nafionbp4
5	PFO2HxA
6	PFO3OA
7	PFO4DA
8	PFO5DoDA
9	PMPA
10	NVHOS
11	PEPA
12	PFBA
13	PFPeA
14	PFHxA
15	PFHpA
16	PFOA
17	PFNA
18	PFDA
19	PFBS
20	PFHxS
21	PFOS
22	6:2_FTS

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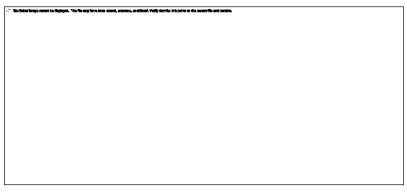

On Tue, Jul 16, 2019 at 3:25 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:
We also are interested in Hydro-Eve and we also have a standard for that.

Seems like we looked for 24, so want Nadine to weigh in, in case I missed one.

Thanks.

On Tue, Jul 16, 2019 at 2:57 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:
Hi Jane,
Thanks I found the document on MENDIX, as you suggested. Are the 22 compounds in that document the ones you are interested in analyzing for in these samples as well (see table below)? Sample prep will be the same, but the main difference between the orbitrap and the QQQ is that you have to decide what analytes you want to look for before running the samples. The QQQ is also inherently more suited to quantitation (most would argue).

Thanks for the heads up on the nomenclature - I thought it was PFAS but saw Wellington refer to their catalog section as PFC so incorrectly altered my language.

Thanks.

1	GenX
2	Nafionbp1
3	Nafionbp2
4	Nafionbp4
5	PFO2HxA
6	PFO3OA
7	PFO4DA
8	PFO5DoDA
9	PMPA
10	NVHOS
11	PEPA
12	PFBA
13	PFPeA
14	PFHxA
15	PFHpA
16	PFOA
17	PFNA
18	PFDA
19	PFBS
20	PFHxS
21	PFOS
22	6:2_FTS

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On Tue, Jul 16, 2019 at 2:41 PM Jane Hoppin <jahoppin@ncsu.edu> wrote:

Hey Jeff,

I'm excited to see you working on this. We already shared our blood protocol with Allison, so you should review that, so you won't be starting brand new. Someone should have shared those with you and you should work with those. I know there will be some differences between the QQQ and the orbitrap, but the sample preparation should be the same.

FYI, we call these PFAS and not PFCs (PFCs include the fluorochemicals that damage the ozone layer).

Please let me know if you need the document I previously sent Allison. I thought she was going to upload into Mendix

Thanks.

Jane

On Tue, Jul 16, 2019 at 2:35 PM Jeffrey Enders <jrenders@ncsu.edu> wrote:

Hi Jane and Nadine,

Dave and I have met and I will begin working on a PFC method for your blood samples. I don't have much information on which compounds you are primarily interested in and this will have a significant impact on the time, effort requirement, and feasibility of this study. I have been collecting information from folks about what standards we have, what methods we have already developed, and what protocols have already been written up. I will try to summarize what is available and try to get from your which compounds you are hoping to quantify.

I will primarily be building off of protocols that Detlef's lab already runs and an instrument method that was shared by Duke and has been partially set up on our instrument. The protocol that Zack Hopkins has shared with me lists the following compounds as being detectable:

Chemical Name	Acronym	Isomer	Internal Standard
Perfluoro-n-butanoic acid	PFBA	linear	MPFBA
Perfluoro-n-pentanoic acid	PFPeA	linear	M5PFPeA
Perfluoro-n-hexanoic acid	PFHxA	linear	M5PFHxA
Perfluoro-n-heptanoic acid	PFHpA	linear	M4PFHpA
Perfluoro-n-octanoic acid	PFOA	linear	M8PFOA
Perfluoro-n-nonanoic acid	PFNA	linear	M9PFNA
Perfluoro-n-decanoic acid	PFDA	linear	M6PFDA
Perfluoro-n-undecanoic acid	PFUnDA	linear	M7PFUnDA
Perfluoro-n-dodecanoic acid	PFDoDA	linear	MPFDoDA
Perfluoro-n-tridecanoic acid	PFTrDA	linear	N/A – use M2PFTeDA
Perfluoro-n-tetradecanoic acid	PFTeDA	linear	M2PFTeDA
Perfluorobutane sulfonate	PFBS	linear	M3PFBS
Perfluoropentane sulfonate	PFPeS	linear	N/A – use M3PFHxS
Perfluorohexane sulfonate	PFHxS	linear / branched	M3PFHxS
Perfluoroheptane sulfonate	PFHpS	linear	N/A – use M3PFHxS
Perfluorooctane sulfonate	PFOS	linear / branched	M8PFOS
Perfluorononane sulfonate	PFNS	linear	MPFNS
Perfluorodecane sulfonate	PFDS	linear	MPFDS
Perfluorooctane sulfonamide	PFOSA	linear	M8FOSA-I
N-methylfluorooctance sulfonamido acetic acid	N-MeFOSAA	linear	d3-N-MeFOSAA
N-ethylfluorooctance sulfonamido acetic acid	N-EtFOSAA	linear	d5-N-EtFOSAA
4:2 fluorotelomer sulfonate	4:2 FTS	linear	M2-4:2 FTS
6:2 fluorotelomer sulfonate	6:2 FTS	linear	M2-6:2 FTS
8:2 fluorotelomer sulfonate	8:2 FTS	linear	M2-8:2 FTS

If we stick to the first table alone, the method development step will progress much more quickly as these compounds are sold by Wellington as a mixture and so can easily be made into a calibration curve. The second table is made manually by adding all compounds one at a time and so this will increase complexity. All of the compounds in these two tables are in the method that we are working to set up on the instrument. Additionally, Wellington and Cambridge isotope labs sell additional PFC compounds. There are far too many to list here but the links can be found below:

- https://well-labs.com/wellingtoncatalogue1618.html (starting on page 140)
- https://shop.isotope.com/category.aspx?id=10032748

Adding compounds to the method beyond the tables listed in this email, while possible, will increase the complexity of the method and inherently increase the risk of internal interferences (i.e., one compound enhances or suppressed the signal of another compound in method). The method that the Knappe group use is about 20 min long and adding compounds may also necessitate making this method longer due to instrument scan speed issues.

Any information you have on compounds of interest or other thoughts or concerns would help guide the conversation. I'm looking forward to working with you on this. Thanks!

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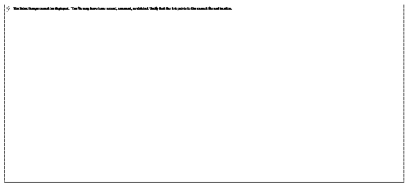

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